CcO by electrochemistry. The advantage of this system is that modeling studies can be directly compared with actual measurements using a combination of electrochemical and spectroscopic techniques. Previous modeling studies of fast-scan voltammetry and time-resolved FTIR studies have shown that ET from the electrode into the enzyme highly likely occurs via CuA. Thereafter electrons are transferred to the remaining redox centers in exactly the same sequence that is natural to the enzyme. In the present investigation we extend this four-electron transfer model in two steps. In the first step we consider protonation equilibria of the oxidized and reduced species for each of the four centers. In the second step we add oxygen/H2O as the terminal (fifth) redox couple including protonation of reduced oxygen to water. Finally we arrive at a kinetic model comprising five redox couples describing a string of bimolecular reactions with protonations. Protons pumped by the enzyme arrive at the electrode, where they are reduced to molecular hydrogen. Hence both electron and proton currents can be observed. Different parameter settings are employed for the modeling, in which known standard redox potentials of the redox centers are maintained while protonation constants are varied. The model is shown to simulate cyclovoltammetry data of the CcO in the absence and presence of oxygen.

2920-Pos Board B690

Interfacial Proton Diffusion

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Lateral diffusion along the membrane represents the most efficient pathway for proton transport between membrane proteins. The recent observation that protons do not jump between ionizable residues but travel along interfacial water molecules (A. Springer et al., PNAS, 2011) suggested that membrane lipids are not required and that lateral proton diffusion may be observed adjacent to any hydrophobic surface. To test that prediction we measured surface proton diffusion in a minimalistic system at the water-decane interface. Although the interface did not offer any proton binding sites, lateral proton diffusion occurred. Fitting a mathematical model to the proton density in the observation spot suggested that proton diffusion was decoupled from bulk at low buffer capacity. At higher aqueous buffer concentrations and in heavy water, proton bulk diffusion became more important. This proves that the retarded proton surface to bulk release is (i) a fundamental property of the boundary between hydrophobic and hydrophilic phases, and (ii) independent on the presence of specific lipids or amino acids.

2921-Pos Board B691

Tuning the Midpoint Potential of the Primary Quinone in Rhodobacter Sphaeroides Photosynthetic Reaction Centers by Mutation of Residue M265

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The photosynthetic reaction center from *Rhodobacter sphaeroides* tunes the midpoint potential (E_m) of its quinones to allow favorable electron transfer from the primary (Q_A) to secondary (Q_B) quinone. Previous work showed that mutation of isoleucine residue 265 of the M subunit (Ile^{M265}) to the polar residues serine or threonine lowers the E_m by 80 or 100 mV, respectively [1]. We have now solved the x-ray crystal structures of these mutants, and the structures do not support the proposal that the E_m drop is caused by lengthening of hydrogen bonds to the quinone. Instead, we hypothesize that the major contribution comes from change to the electrostatic potential at the Q_A site, and we have characterized additional mutants at this site. Mutation of Ile^{M265} to asparagine shows a faster Q_A back reaction (k_P^A=19 s^{-1} at pH 8.0) and slower Q_B back reaction (k_P^B=0.12 s^{-1} at pH 8.0) than wild type, but similar to the threonine mutant. The estimated equilibrium potential between Q_A and Q_B (K_{AB}) for the asparagine mutant is 156, essentially the same as the threonine mutant (157). However, the Ile^{M265} to glutamine mutant has a slower k_P^A (6.3 s^{-1} at pH 8.0) and a slower k_P^B (0.51 s^{-1} at pH 8.0) than wild type. The estimated K_{AB} of the glutamine mutant is similar to the wild type (11 vs. 10, respectively), indicating no significant change in E_m of Q_A. The anomalous behavior of the glutamine mutant may be due to restricted freedom of rotation of the longer glutamine side chain, likely clashing with the quinone and preventing adoption of a conformation with an electrostatic minimum energy.

[1] Wells et al. (2003) *Biochemistry* 42, 4064-4074

2922-Pos Board B692

Kinetics of Proton Release and Uptake by Channelrhodopsin-2

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Neurophysiological experimentation has recently been revolutionized when the algal membrane protein channelrhodopsin-2 (ChR2) was introduced as an optical tool to elicit action potentials by light. However, the functional mechanism of ChR2 is still obscure and the difficulties to resolve cation channeling on the molecular level were recently complicated by the finding that ChR2 also exhibits proton pumping activity. To correlate the channeling and pumping activities, we determine here the kinetics of proton release and uptake by ChR2 using the pH-indicating dye bromoxylenol blue. We find that proton release precedes proton uptake despite the fact that ChR2 lacks the corresponding residue to E204 of bacteriorhodopsin (BR). The S245E mutant, designed to reinstall the supposedly crucial carboxylic side chain for early proton release, does not exhibit any functional differences to wild-type ChR2. Surface-to-bulk proton transfer is not rate-limiting to the response of the water-soluble pH-indicator as demonstrated by experiments in the presence of mobile buffer. The kinetics of proton release and uptake by ChR2 match the rise and decay of the P520 intermediate. As the P520 state also represents the conductive state for cation channeling, the temporal concurrence of proton pumping and channel gating remains enigmatic.

2923-Pos Board B693

Design and Characterization of a Multi-Cofactor Binding Protein with Implications for Photoactivated Water Oxidation

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The potential lies in solar energy to power all human activities with minimal environmental consequences. The challenge we face is in the development of catalysts for solar energy conversion that are efficient and cost-effective. Inspired by the structure and function photosystem II, progress has been made toward developing catalysts that use sunlight to oxidize water to oxygen and hydrogen, a high-energy fuel. Here, we begin a new approach to light-driven water oxidation in which designed proteins will be the catalysts. We report the first *de novo* designed protein capable of conducting photoactivated charge separation. It is a 4-α-helix bundle that uses heme and zinc protoporphyrin IX (ZnPPIX) cofactors to achieve electron transfer upon illumination. This protein was redesigned to extend the electron transfer chain to a di-metal site (Figure 1). The interactions of the proteins with heme, ZnPPIX, and various metals were characterized by circular dichroism and UV/visible spectroscopy. The metal cluster is designed to serve as the water oxidation center of the protein to recreate the function of the oxygen-evolving Mn_4CaO_5 center in photosystem II.

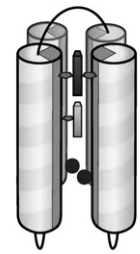

Figure 1: Structure of designed holo-protein. Cofactors from top to bottom: heme, ZnPPIX, di-metal center.

2924-Pos Board B694

Bioluminescence of Scintillons Isolated from *Noctiluca Miliaris* is Inhibited by Divalent Metal Cations, Suggesting Proton Channel Involvement

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Fogel and Hastings (1972, *P.N.A.S.* USA 69:690-693) first hypothesized the existence of voltage gated proton channels in bioluminescent dinoflagellates, where they were postulated to trigger the flash. The flash originates in discrete organelles termed scintillons (Nicolas et al., 1987 J. Cell Biol. 105: 723-735.) that contain luciferase and its substrate, luciferin. Luciferase is activated, and luciferin is released from a binding protein, at low pH. Proton channels in the membranes of the scintillon were postulated to conduct H^+ from the acidic central flotation vacuole into the scintillon, activating luciferase and releasing luciferin, causing a bioluminescent flash. We have recently cloned a voltage gated proton channel (kH_V1) from a non-bioluminescent dinoflagellate, *Karlodinium veneficum* (Smith et al., 2011 *P.N.A.S.in-press*). In heterologous expression systems, this channel exhibits a negative $V_{threshold}$ so that inward current is activated, consistent with the proposed role of allowing proton flux from the vacuole into the scintillon. The H^+ current of heterologously expressed kH_V1 is inhibited by Zn^{2+}, but at much higher concentrations than in mammalian channels. We have developed a method to isolate functional scintillons from the bioluminescent species *Noctiluca miliaris* through gentle homogenization and sucrose density gradient centrifugation. The density of the scintillon fraction was found to be 1.213 g/cm^3. Reducing the pH of the scintillon suspension to below pH 6 elicits a luminescent flash. Addition of divalent metals to the suspension diminishes the intensity of the flash. Zn^{2+}, Ni^{2+}, Cu^{2+}, Co^{2+}, and Cd^{2+} at millimolar concentrations abolished the luminescence from isolated scintillons. The sensitivity of scintillon luminescence to divalent metals is consistent with the idea that proton selective channels in the scintillon membrane mediate the activation of the flash.